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MICROX II - A New Generation of Portable Measuring Systems for Microoptodes

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Abstract

Sediments, microbial mats, biofilms and other microbial communities are characterized by steep gradients of physical and chemical parameters[12, 29]. Fiber optical microsensors, microoptodes, that we developed over the last four years have become powerful tools to investigate and measure these parameters with a sufficient spatial resolution and with a minor disturbance of the micro-environment in natural systems[8-11, 15-19].

Together with microoptodes for oxygen[15-17], pH[19], temperature[11, 17] we developed a sensitive measuring system[9, 10] that enables the measurement of luminescence intensities and lifetimes of indicators that are immobilized at the tip of tapered fibers.

As light sources, we used light emitting diodes, that nowadays are available with a high optical power output even in the blue part of the spectrum, where many indicators can be excited. Furthermore, LEDs are easy to modulate and thus enable both, the measurement of luminescence intensities independent of ambient light and the measurement of luminescence lifetimes based on a phase modulation technique. The weak amount of light emitted at the tip of a dip-coated silica-silica fiber required, however, a photomultiplier tube (PMT) as detector[9, 10, 15]. Although the PMTs are very sensitive light detectors, the application in natural systems for measurement of concentration profiles with and without strong ambient light causes additional noise problems with the PMT[10]. Therefore, we improved the composition of the sensing layer that covers the tapered fiber tip and the taper geometry, because both have a large impact on the signal. Furthermore, we improved the optical setup to reduce inherent coupling losses and inherent noise signals like unwanted additional luminescence caused by epoxy, solvents etc..

We present the first all-solid-state instrument, MICROX II, that is based on the previously released portable instrument MICROX I, that uses a PMT as detector. Noise influences caused by additional luminescence, and their possible reduction will be presented. Examples of first applications of the instrument will also be shown.

Introduction

The physiological conditions which controls the life of microbial communities living in marine sediments or the life of organisms in biofilms is to a large extend investigated by microsensor measurements. These microsensors, in our understanding, sensors with tip diameters smaller than 100 µm, enabled the evaluation of the steep changes in solute concentrations or parameters like pH and temperature occurring within hundreds of micrometers[12, 29]. The largest variety of these microsensors are different types of microelectrodes, most of which have to be manufactured in a time consuming process. Since 4 years we have developed a couple of fiberoptical microsensors that are easier to make and show some interesting features making them suitable for applications in marine environment, like long lifetime, negligible dependency on hydrostatic pressure, no stirring sensitivity, no influence by electromagnetic noise and mechanical flexibility. The have proven to be a good supplement to the established family of microelectrodes.

Fiberoptical microsensors - microoptodes

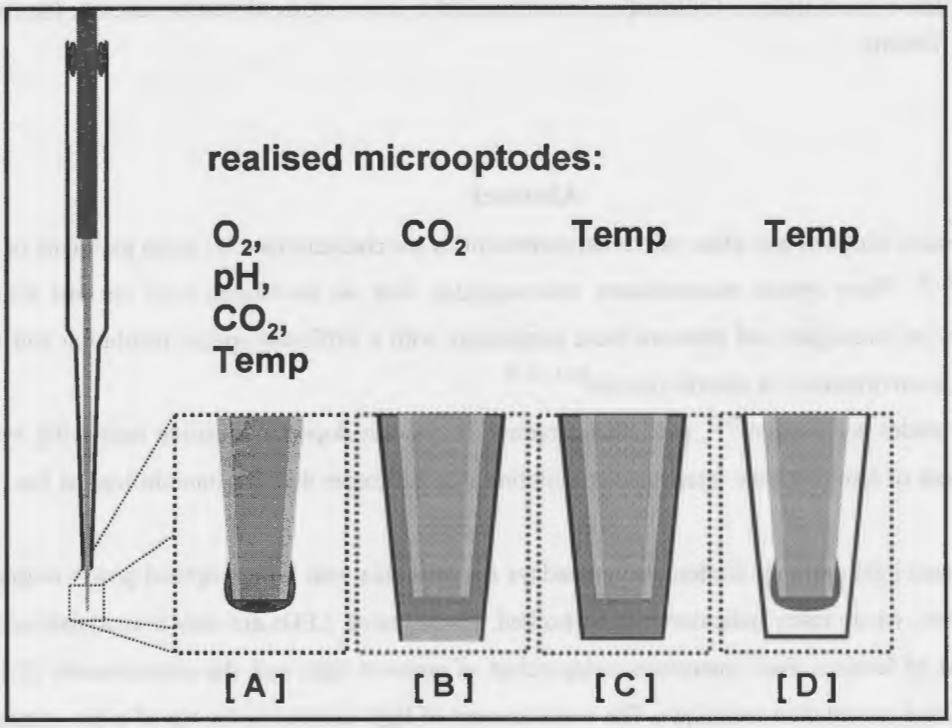

Figure 1: Structural overview of realized microoptodes (schematic drawing):
[A] Dip coated tapered fiber that is fixed in an open microcapillary. The sensing layer is an indicator polymer mixture.
[B] The tapered fiber is inserted in a liquid indicator filled microcapillary, which has been closed at the tip by an analyte permeable membrane.
[C] The tapered fiber is inserted in an indicator filled and closed microcapillary.
[D] The tapered fiber is dip-coated and inserted in a gas filled closed microcapillary.

The basic material of all our microoptodes is a standard silica/silica multimode graded index fiber with a 100 µm core and 140 µm cladding diameter (Radiall, Germany). The fiber is supplied with a standard ST-plug (Radiall, Germany) at the measuring system end. The first steps of the preparation are identical for all microoptodes. At the sensor end, the PVC tube and the plastic jacket are removed. The blank fiber is either tapered by a hot flame, e. g. a gas burner, or by a light arc of a

fusion splicer. Then the taper is cut or broken at a diameter between 10 - 30 µm which turned out to be a good compromise between necessary spatial resolution (the smaller the better) and light output signal (the larger the better).

Now it depends on the type of sensor how the fiber is further processed. Figure 1 shows the different microoptode structures that have been realized. Type [A] (Fig. 1) is the straightforward approach, the taper is dipped into an indicator polymer solution. After evaporation of the solvent, the tip is coated with a sensing layer. For the usual profiling measurement, the flexible fiber is fixed by epoxy in a standard microcapillary that can be handled by various mechanical holders e.g. micromanipulators. Another fixation can be a syringe. By this preparation microoptodes for oxygen[8, 16,17, 30, 35], pH[19], carbon dioxide (unpublished results of O. Kohls) and temperature[11, 17] have been realized. Type [B] (Fig. 1) is a blank tapered fiber, which is inserted in an indicator solution filled microcapillary that is closed by a gas permeable membrane. This is the microoptical version of the so called Severinghaus principle, which is widely applied for electrochemical carbon dioxide sensors (carbon dioxide penetrates the membrane and changes the pH of the indicator solution which in turn changes its optical properties). In our case it is used for the same purpose and enabled the first carbon dioxide profiles from 4000m water depth (unpublished results of O. Kohls, E. Epping and F. Wenzhöfer). Type [C] is very similar to type [B] only that the microcapillary is closed, so the tip is gas non permeable. This structure gave the temperature sensor with the highest temperature resolution[11]. Type [D] (Fig. 1) is another realization for temperature microoptodes[11]. Here the taper is dip-coated like type [A] but additionally inserted in a closed, air filled microcapillary. Additionally the pure tapered fiber can be used for simple backscatter measurements which e.g. can detect the point where a fiber penetrates the surface of a sediment[18]. But this is more a microprobe than a microoptode and not added to the structural overview in figure 1. The principles behind the optical detection of the described parameters: oyxgen[1-4, 6, 13-15, 21-23, 28, 30, 31, 33, 34, 36], pH[19, 25, 27, 32, 33, 36], carbon dioxide[24, 26, 33, 36] and temperature[5, 7, 11, 36, 37] are well described in literature. According to these principles most of which are luminescence measurements, the necessary measuring system should be able to measure both, luminescence intensities and luminescence lifetimes. A task that can be accomplished by a dual-phase lockin detection principle that enables the application of phase modulation techniques[4,20] as well as sensitive intensity measurements that are independent of the ambient light level.

MICROX 1 & 2 measuring system

Optical coupling of microoptodes

To solve the general problem of using a single strand silica/silica fiber as optical microsensor that has to be connected to a measuring system there exist three possible coupling methods, that are shown in figure 2.

The classical way is a beamsplitter setup (Fig. 2 [A]) in which a semi-reflective mirror is used to couple 50% of the excitation light (Fig. 2 ex) into the fiber. Fifty percent of the signal light reaches via the same mirror the detector (Fig. 2 det). The transmission can be improved if a dichroic mirror can be used (for luminescence measurements with a large Stoke's shift). The setup is simple by its structure and can give high signals in the case of a dichroic mirror. But a very good blocking efficiency of the optical filters is necessary because large amounts of excitation light may reach the detector by direct reflections in the setup. Additional background signals may arise because of unexpected luminescence of materials that are currently used to make fiberoptical plugs and their fixation glues as will be shown later. Furthermore, each part of the beamsplitter setup needs a relatively high level of adjustment (see table 1).

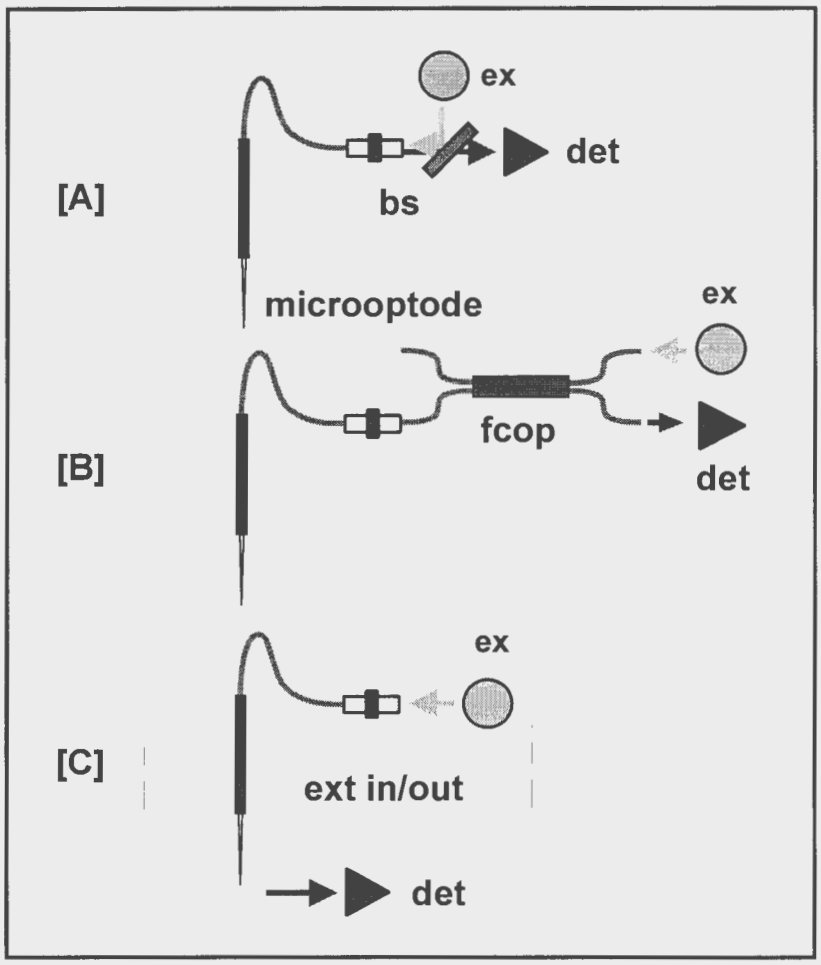

Figure 2: Possible methods of optical coupling for microoptodes (schematic drawing):
[A] Classical beamsplitter (bs) setup - the position for in and out coupling of excitation and emission light can be exchanged, the performance can be improved if a dichroic mirror can be used.
[B] Fiber coupler (fcop) setup - the 2x2 fiber coupler is a standard device in telecommunications technology, here are 50% signal loss with every transmission.
[C] External in or out coupling (ext in/out) setup - here the lightguiding property of the microsensor is only used for one direction, either excitation or emission, and is therefore restricted to the whole experimental setup.

The second possible method are fiber couplers (Fig. 2 [B]), e.g. 2x2 melting couplers or grinded couplers or as currently released, GRIN lense couplers (Fig. 2 fcop)[8-11, 15-19]. These are standard products of the telecommunications industry and if they are once supplied with standard optical fiber plugs, their way of connection is simple, with a low level of adjustment. Like the beamsplitter they have 50% attenuation in both directions. Although they might luminesce because of the fixation glue used to fix the mechanical shield around the melted part of the two fibers, the optical filters in the system do not need such a high quality because the excitation light is only guided indirectly to the detector reflected from the coupler and the fiber endfaces (see table 1). The last method is the external in or out-coupling of light (Fig. 2 [C]). In this method the fiber of the microoptode is used for one way of light only, that means either the excitation light is launched into the fiber, which is shown in figure 2, and the light signal is collected externally[30, 32], or the fiber tip is illuminated externally and the light signal is guided through the fiber to the detector. This method allows the use of fiber tips smaller than 1 µm in diameter

that enable measurements even in single cells. For flexible applications in biofilms or sediments like the above described, however, this method is not useful, because it is extremely confined to a special fixed setup (table 1).

Table 1

comparison of optical coupling methods for microoptodes		
coupling	**pro**	**con**
beam splitter, dichroic beam splitter	• simple setup • with dichroic mirror higher signal transmission	• good blocking efficiency of optical filters is mandatory • high level of adjustment • high background "noise"
fiber coupler	• simple adjustment with standard equipment	• high signal loss (50%) • fragile setup
external in- or out-coupling	• smallest sensor tip (< 1µm possible)	• restricted to fixed setup • no flexible application of microoptode

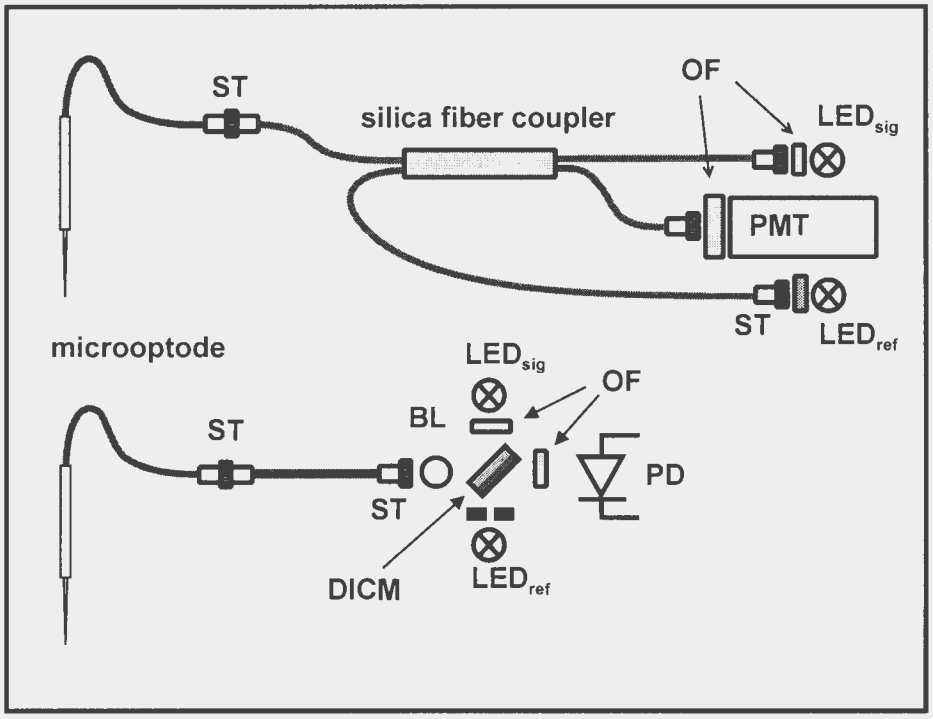

Figure 3: Optical setups of the measuring systems MICROX 1 & 2 (schematic drawing):
MICROX 1: The excitation light (light emitting diode, LED_{sig}) is launched via an excitation filter (OF) and a standard fiber connector (ST) into one branch of a fiber coupler (silica fiber coupler). The microoptode is connected to one branch of the coupler on the left side via standard fiber connectors (ST). The emitted light of the sensor tip is guided back and reaches via the second branch on the right side, via fiber connector (ST) and emission filter (OF) the photodetector (photomultiplier tube, PMT). The reference light source (a red light emitting diode, LED_{ref}) uses the second branch on the left side to couple in its light to the signal path.
MICROX 2: The excitation light (light emitting diode, LED_{sig}) is launched via an excitation filter (OF), a dichroic mirror (DICM), a ball lense (BL) and a standard fiber connector (ST) into an adapter fiber (adfib) which is connected to the microoptode. The emitted light of the sensor tip is guided back and returns by the same path to the dichroic mirror where it is transmitted. Then the light reaches via an emission filter the photodetector (photodiode, PD). The reference light (a red light emitting diode, LED_{ref}) passes a small aperture and is reflected on the back surface of the dichroic mirror onto the detector.

Optical coupling of MICROX 1 & 2

The MICROX 1 used a 2x2 fiber coupler[8-11, 15-19] (Fig. 3 silica fiber coupler) to connect the microoptode to the measuring system. Because of the weak light signal which usually was in the range of 10-50 pW, a photomultiplier tube (PMT) was used as detector (photosensor module, Hamamatsu Photonics, Germany). The coupler (Gould, USA) was chosen because of its availability as standard device, the simple adjustment and its low background signal. The second left branch of the coupler (Fig. 3) was used to couple in light of a red LED (Fig. 3 LED_{ref}) alternately to the excitation light (Fig. 3 LED_{sig}) to obtain a reference signal that is necessary for evaluating the zero phase angle of the system for the phase modulation technique. While we further improved the light output of the oxygen and temperature microoptodes (range of 50-200 pW), we developed our own optimized optical setup that is based on a dichroic mirror (Fig.3 DICM) that reflects the blue excitation light (Fig. 3 LED_{sig}) and transmits the red emission light. A ball lense is used to couple the light into and out of the fiber efficiently, while the optical filters were carefully selected to optimize the blocking efficiency.

As already mentioned, in this type of setup there might occur severe sources of background signals that we did not expect. Table 2 shows results of measurements with this optical setup that were measured with non modulated light and an excitation LED current of 20 mA.

Table 2

additional luminescence in the dichroic beamsplitter setup – MICROX 2		
connected to	**measured photocurrent [pA]**	**signal**
empty	8	background of the optical setup
fiber plug (ceramics ferrule)	51	luminescence of white ceramics
fiber plug + fiber (ceramics ferrule)	460	luminescence of white ceramics + fiber fixation epoxy
fiber plug + fiber + microoptode (ceramics ferrule)	1870	large signal with large background
fiber plug + fiber (steel ferrule, crimped fiber)	22	background signal of insufficient blocking of excitation light
fiber plug + fiber + microoptode (steel ferrule, crimped fiber)	1080	medium signal with low background

The situation „empty" deliver the background signal of 8 pA due to reflections and insufficient blocking within the setup. If a standard fiber plug with ceramics as material of the ferrule without a fiber is used there appear two more components in the background signal of 51 pA. More reflected excitation light from the front face that is insufficiently blocked and additional luminescence from the whiteners in the ceramics reach the detector. If now the same type of plug with inbuild fiber is connected, the background signal rises to 460 pA due to the epoxy, that is used for fixation of the fiber in the plug. Finally, if a microoptode is connected, this gives an overall signal of 1870 pA, which is a large signal on 25% background. To improve that ratio we used another material for the ferrule and another way of fiber fixation. We found the cheap way of fiber connectors, the so called crimp-and-cleave connection (Radiall, Germany). These fiber plugs have steel ferrules and the fiber is broken not polished and hold by plastic and a metal tube that is crimped onto the fiber. With such a plug and inserted fiber the background due to increased excitation light reflection on the surfaces was 22 pA. Because of the less defined position of the fiber and the weaker quality of the surface of the broken fiber, there is a smaller coupling efficiency of these connections which can be seen in the lower overall signal of 1080 pA if the same microoptode is connected at the other end of this adapter cable (Fig. 3). But now the background of 2% is more favorable and is smaller than the

electronical background. With this setup it was possible to realize a MICROX system with a cheap and simple photodiode (Fig. 3 PD) (Hamamatsu Photonics, Germany).

Phase angle detection

The phase modulation technique as method to evaluate the luminescence lifetime has been used in many applications[4, 9, 10, 37]. It is based on a sinusoidal modulation of the excitation light at the modulation frequency f_{mod}. Accordingly the emitted luminescence is sinusoidal but delayed compared to the excitation light. This delay is the phase angle Φ_{sig} that can be measured and is related to the luminescence lifetime τ_{sig} (if a monoexponential luminescence decay curve can be assumed):

$$\tan\left(\Phi_{sig}\right) = 2\pi \cdot f_{mod} \cdot \tau_{sig}$$

Usually the frequency is chosen such as the dynamic range of the phase angle is centered around the 45° value, because this gives the largest change of phase angle to a given change in lifetime[10]. One of the most sensitive detection schemes for amplitude modulated signals is the dual phase lockin principle. The detected sinusoidal signal is multiplied by a sinus- and a cosinus-signal at the same frequency. Afterwards the signals are lowpass filtered which give two signals that are proportional to the amplitudes of the measured signal and the reference signal multiplied by the sinus or the cosinus of the phase angle. The necessary information of phase angle and amplitude can easily be resolved by the following two equations:

$$\Phi_{sig} = \arctan\left(\frac{a_{sig} \cdot \sin\left(\Phi_{sig}\right)}{a_{sig} \cdot \cos\left(\Phi_{sig}\right)}\right)$$

$$a_{sig} = \sqrt{\left(a_{sig} \cdot \sin\left(\Phi_{sig}\right)^2 + \left(a_{sig} \cdot \cos\left(\Phi_{sig}\right)\right)^2\right)}$$

This also clarifies why the dual phase lockin is suitable for detecting both, the phase angle Φ_{sig} and the amplitude a_{sig}. If the lifetime is very short, the phase angle approaches zero and the measured amplitude directly corresponds to the luminescence intensity. To reference the time delays caused by the electronical circuits the phase angle signal is processed in a time multiplex mode. Alternately the excitation LED_{sig} and the reference LED_{ref} (Fig. 3) are switched on and for each case a phase angle is measured. By subtracting them, all influences of the signal path and the detector are canceled and the resulting difference phase angle is the wanted measuring signal. The negligible difference between both types of LEDs is not referenced but can be assumed as constant.

MICROX 1 & 2 - instrument

The inner structure of the instrument can be divided into the following units:

- frequency generation and LED driving circuit
- dual phase lockin
- microcontroller with LCD display, keypad, serial interface
- power supply
- PMT-voltage control and safety switch off circuit (only MICROX 1)
- photodiode preamplifier (only MICROX 2)

- optoelectronical setup (different for MICROX 1 & 2)

All settings and parameters are software controlled by the microcontroller and can be accessed by the keypad. In the measuring subprogram the data are constantly send via the serial interface to a PC, that stores and displays the data in different modes. The instrument connected to a Notebook PC can be seen in figure 4.

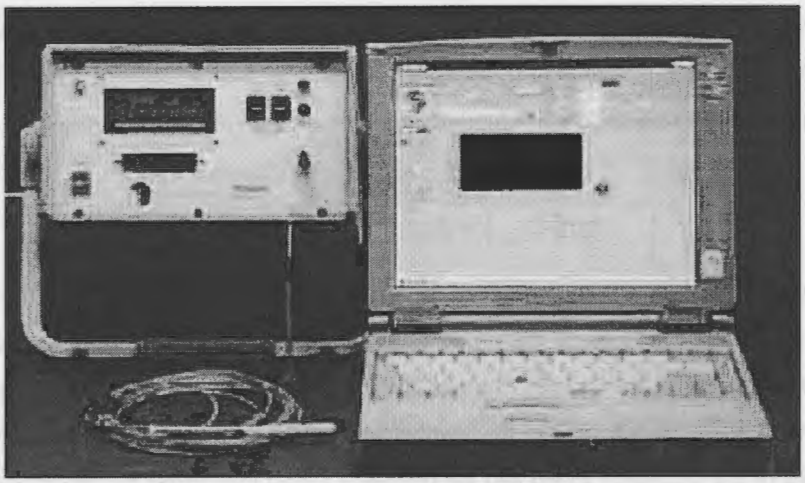

Figure 4: Photo of a MICROX 1 measuring system connected to a Notebook PC with the MICROX software that displays and stores the data, which are delivered by the instrument. The cable in front of the instrument is an oxygen microoptode connected to the device. The MICROX 1 is software controlled via the front panel keypad, while all necessary informations of adjustable parameters and the measured data are shown on the LCD-display.

Errors in phase angle detection

Concerning errors in the phase angle measurement we distinguished three principal error categories that might have various sources:

1. Additional background signal that has the same modulation frequency and adds to the real luminescence signal which can be caused by:
    - background luminescence of optical system
    - electronical coupling via power supply in high gain amplifier circuits
    - externally excited natural luminescence e.g. chlorophyll
2. An offset phase angle which can be caused by:
    - different phase angle situation of excitation and reference LED
    - total signal dependent phase angle situation of photomultiplier tube
    - unsymmetry in sinus and cosinus signals
3. Error in measuring the phase angle which can be caused by:
    - noise of each part of the signal path, most dominant the photodetector
    - bandwidth of the dual phase lockin

If the first two sources of background signals stay constant during the measurement, they can be corrected which is shown in the next chapter. The chlorophyll luminescence, which varies with the biological system, is not constant in a profiling application. This can be canceled by an additional optical insulation on the sensor tip or an additional optical cutoff filter in front of the photodetector, which is the case for the MICROX system. The difference phase angle of the two LEDs has

minor importance, while the behavior of the PMT if it catches large amounts of ambient light via the microoptode (microoptode without optical insulation and measurement with illuminated probe, sun light conditions) can only be solved by an optical insulation (which increases the sensor response time) or by replacing the PMT by a photodiode that does not show this behavior. This has been done in the MICROX 2 compared to MICROX 1. The error in the measurement is a general optimization issue and relies on the signal-to-noise-ratio which has been optimized by increasing the efficiency of the optical setup and by optimizing the sensing chemistry that give a higher light output.

Phase angle correction for constant background signal

If the background signal is constant during the measuring time and if it can be measured separately, e. g. by leaving the instrument unconnected, it is possible to correct the measured, „false" data. The measured sinusoidal signal $s_{meas}(t)$ can be described as follows:

$$s_{meas}(t) = DC_{meas} + a_{meas} \cdot \sin(2\pi f_{mod} t - \Phi_{meas})$$

($s_{meas}(t)$ = measured light signal, DC_{meas} = measured DC signal, a_{meas} = measured amplitude, f_{mod} = modulation frequency, Φ_{meas} = measured phase angle)

Furthermore it can be assumed that the measured signal $s_{meas}(t)$ is the result of a linear combination of two sinusoidal signals,

$$s_{meas}(t) = s_{sig}(t) + s_{nois}(t)$$

the „real" luminescence signal $s_{sig}(t)$:

$$s_{sig}(t) = DC_{sig} + a_{sig} \cdot \sin(2\pi f_{mod} t - \Phi_{sig})$$

($s_{sig}(t)$ = luminescence light signal, DC_{sig} = luminescence DC signal, a_{sig} = luminescence amplitude, Φ_{sig} = phase angle caused by luminescence lifetime)

and the background or noise signal $s_{nois}(t)$:

$$s_{nois}(t) = DC_{nois} + a_{nois} \cdot \sin(2\pi f_{mod} t - \Phi_{nois})$$

($s_{nois}(t)$ = background signal, DC_{nois} = background DC signal, a_{nois} = background amplitude, Φ_{nois} = phase angle caused by background signal)

It does not matter whether the background signal is a luminescence signal of the optical system or it is an electronically coupled signal, it only has to be constant during the measurement. If it changes, like natural external additional luminescence does, it can not be corrected by a single frequency measurement.

By comparison and the use of trigonometric theorems, it can be derived that the luminescence lifetime induced phase angle is:

$$\Phi_{sig} = \arctan\left(\frac{a_{meas} \cdot \sin(\Phi_{meas}) - a_{nois} \cdot \sin(\Phi_{nois})}{a_{meas} \cdot \cos(\Phi_{meas}) - a_{nois} \cdot \cos(\Phi_{nois})}\right)$$

while the luminescence amplitude is:

$$a_{sig} = a_{meas} \cdot \frac{\sin(\Phi_{meas})}{\sin(\Phi_{sig})} - a_{noi} \cdot \frac{\sin(\Phi_{nois})}{\sin(\Phi_{sig})}$$

These correction formula are implemented in the MICROX systems and have shown to give good results if the noise signal does not exceed 70% of the real signal. We think that this constraint is due to a principle signal to noise problem and if this situation occurs consider the sensor as being damaged.

Experimental setups & results

The first experimental setup should investigate, if the new system, MICROX 2, with its photodiode as photodetector shows the same relation between increasing non-modulated, so called DC-light and an increase in offset phase angle and measuring noise, as the photomuliplier tube based MICROX 1 has shown. Therefore an optically non-insulated microoptode has been positioned in a glass filled with aerated water. At the bottom of the glass was a 10 mm thick layer of glass spheres in a size range up to 100 µm in diameter. The sensor tip was positioned 2 mm above the surface of the spheres. Outside the glass the two arms of a swan-neck lamp were adjusted that maximum light was coupled on the glass sphere surface. The phase angle was stored with MICROX 1 and 2, at different light levels over a time period of 70 seconds.

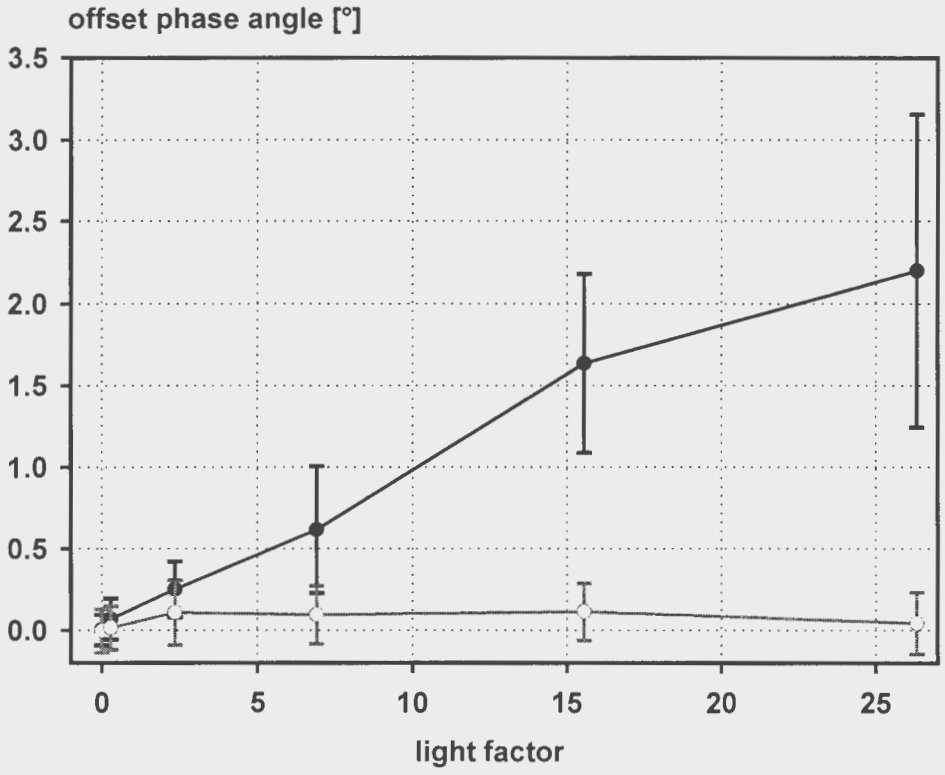

Figure 5: The two plots demonstrate the reaction of the measuring systems MICROX 1 & 2 to an increase of additional light that is coupled into the measuring system via the sensor tip (offset phase angle vs. light factor). The black curve (filled symbols) shows the result of MICROX 1 and the grey curve (open symbols) shows the result of MICROX 2.

To generate a comparable index for the creation of a graph, we defined the light factor LF:

$$LF = \frac{DC_{tot} - DC_{sig}}{a_{sig}}$$

It is made of the total measured non-modulated part of the light, DC_{tot}, subtracted by the luminescence part, DC_{sig}, of it. The result is divided by the signal amplitude a_{sig}. Therefore LF describes how much higher the ambient light signal is compared to the luminescence signal. The results are shown in figure 5. Both curves a related to the start value in darkness because there was a general offset phase angle of $\Phi_{off} = 1.1$ ° between the measurements of MICROX 1 and 2 which is due to different types of reference LEDs used in the instruments. In the darkness the MICROX 1 exhibits a lower noise (smaller error bar) compared to the MICROX 2. If the light factor increases, an offset phase angle occurs in the MICROX 1 curve, which increases and the noise increases as well which can be seen in the error bars. The MICROX 2 curve does not show a significant change and the noise stays constant. As the whole signal processing behind the optical setup, the photodetector and the preamplifiers is the same, this is a strong indication for the different behavior of photomultiplier tubes and photodiodes (incl. preamplifiers) if they have to face large levels of non-signal light.

The second application is the standard application of microoptodes, the measurement of penetration depth profiles of parameters like oxygen, temperature, etc.. A sample sediment core of an intertidal North Sea sediment was placed in a flow chamber and was constantly flushed with aerated salt water at 10 °C. Oxygen profiles were measured in the darkness and with illumination of the setup with the same sensor like above. The darkness curve (Fig. 6) shows the typical decrease of oxygen down to zero at a depth of 2 mm as has been measured with microelectrodes by colleagues.

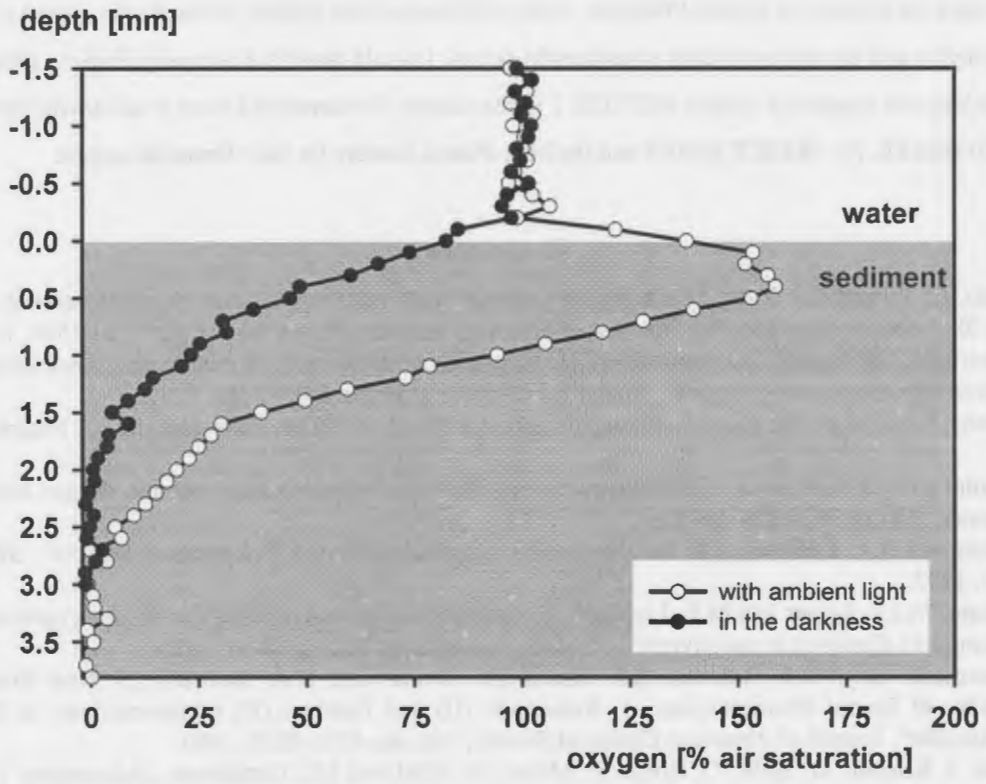

Figure 6: These oxygen profiles (penetration depth [mm] vs. oxygen concentration [% air saturation]) were measured in a sample core of an intertidal North Sea (Wadden Sea) sediment. One profile was measured with the sample being illuminated (open symbols) and one profile was measured in the darkness (filled symbols).

When the sample is illuminated the photosynthetically active organisms become productive as can be seen in the oxygen peak of the light profile (Fig. 6) at 500 µm depth in the sediment and the deeper penetration of oxygen down to 2.7 mm. Many of these measurements in such a same sample usually are used to evaluate exchange rates, metabolism of the bacteria and algae that settle these sediments. The slight zig zag deviations at some depths can be either due to biological reasons, e.g. bioturbation, wormholes, but also can be due to the still high noise of the MICROX 2 which has to be improved.

Finally the MICROX 2 has a faster warm up time of less than 5 minutes while the MICROX 1 has about 30 minutes. Both instruments can be powered by 110 - 240 VAC or by 9-36 VDC which makes them suitable for field applications. The power consumption is 4.3 W.

Summary

The first luminescence lifetime based measuring system for microoptodes, MICROX 1, has been optimized by further improving the optical setup for coupling the microoptodes and further improving the microoptodes concerning light output. This resulted in the first all solid state measuring system for microoptodes, MICROX 2, which showed an improved performance if microoptode measurements should be executed with optically non insulated sensors in environments with high levels of ambient light. In the MICROX 2 the phase angle offset and phase angle noise show no dependence of the light levels as it has been found with the MICROX 1. Furthermore it has a much faster warm up time. However, if the optimized optical setup, that has an inherent spectral restriction with the dichroic mirror, can be used for pH and carbon dioxide sensors, has to be investigated.

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References

[1] J.R. Alcala, C. Yu and G.J. Yeh, „Digital phosphorimeter with frequency domain signal processing: Application to real-time fiber-optic oxygen sensing", Review of Scientific Instrumentation, 64 (6), pp. 1554-1560, 1993.

[2] J.R. Bacon and J.N. Demas, „Determination of oxygen concentrations by luminescence quenching of a polymer immobilized transition-metal complex", Analytical Chemistry, 59, pp. 2780-2785, 1987.

[3] I. Bergman, „Rapid response atmospheric oxygen monitor based on fluorescence quenching", Nature, 218, pp. 396, 1986.

[4] K.W. Berndt and J.R. Lakowicz, „Electroluminescent lamp-based phase fluorometer and oxygen sensor, Analytical Biochemistry, 201, pp. 319-325, 1992.

[5] J.N. Demas and B.A. DeGraff, „On the Design of Luminescence Based Temperature Sensors", SPIE Vol. 1796, pp. 71-75, 1992.

[6] P. Hartmann, M.J.P. Leiner and M.E. Lippitsch, „Luminescence quenching behavior of an oxygen sensor based on a Ru(II) complex dissolved in polystyrene", Analytical Chemistry, 67, pp. 88-93, 1995.

[7] B.L. Hauenstein (Jr.), W.J. Dressick, S.L. Buell, J.N. Demas and B.A. DeGraff, „A New Probe of Solvent Accessibility of Bound Photosensitizer. 1. Ruthenium (II) and Osmium (II) photosensitizers in Sodium Lauryl Sulfate Micelles", Journal of American Chemical Society, 105, pp. 4251-4255, 1983.

[8] R.N. Glud, I. Klimant, G. Holst, O. Kohls, V. Meyer, M. Kühl und J.K. Gundersen, „Adaptation, Test and in situ Measurements with O_2 Micropt(r)odes on Benthic Landers", Deep Sea Research, (in press), 1997.

[9] G. Holst, M. Kühl and I. Klimant, „A Novel Measuring System for Oxygen Microoptodes Based on a Phase Modulation Technique", SPIE Chemical, Biochemical and Environmental Fiber Sensors VII, 2508, pp. 387-398, 1995.

[10] G. Holst, R.N. Glud, M. Kühl and I. Klimant, „A Microoptode Array for Fine Scale Measurement of Oxygen Distribution", Sensors and Actuators B, 38, pp. 122-129, 1997.
[11] G. Holst, M. Kühl, I. Klimant, G. Liebsch und O. Kohls, „Characterization and Application of Temperature Microoptodes for Use in Aquatic Biology", SPIE Advances in Fluorescence Sensing Technology III, 2980, pp. 164-170, 1997.
[12] B.B. Jørgensen and N.P. Revsbech, „Colorless Sulfur Bacteria, Beggiatoa spp. and Thiovolum spp. in O_2 and H_2S microgradients", Applied and Environmental Microbiology, 45, pp. 1261-1270, 1983.
[13] A. Juris, V. Balzani, S. Campagna, P. Belser and A. von Zewelsky, „Ru(II) Polypyridine Complexes: Photophysics, Photochemistry, Electrochemistry and Chemiluminescence", Coordination Chemistry Reviews, 84, pp.85-227, 1988.
[14] H. Kautsky, „Quenching of luminescence by oxygen", Transactions of Faraday Society, 35, pp. 216-219, 1939.
[15] I. Klimant, V. Meyer and M. Kühl, „Fiber-Optic Oxygen Microsensors, a New Tool in Aquatic Biology", Limnology and Oceanography, 40 (6), pp. 1159-1165, 1995.
[16] I. Klimant, G. Holst and M. Kühl, „Oxygen microoptrodes and their application in aquatic environment", SPIE Chemical, Biochemical and Environmental Fiber Sensors VII, 2508, pp. 375-386, 1995.
[17] I. Klimant, M. Kühl, R.N. Glud und G. Holst, „Optical Measurement of Oxygen and Temperature in Microscale: Strategies and Biological Applications", Sensors and Actuators B, 38, pp. 29-37, 1997.
[18] I. Klimant, G. Holst and M. Kühl, „A Simple Fiber-Optic Sensor to Detect the Penetration of Microsensors into Sediments and Other Biological Materials", Limnology and Oceanography, 42 (7), 1997.
[19] O. Kohls, I. Klimant, G. Holst und M. Kühl, „Development and Comparison of pH Microoptodes for Use in Marine Systems", SPIE, 2978, pp. 82-91, 1997.
[20] J.R. Lakowicz, „Principles of Fluorescence Spectroscopy", Plenum Press, New York and London, 3rd. edn., 1983.
[21] M.E. Lippitsch, J. Pusterhofer, M.J.P. Leiner and O.S. Wolfbeis, „Fiber-optic sensor with the fluorescence decay time as the information carrier", Analytical Chemistry, 205, pp. 1-6, 1988.
[22] D.W. Lübbers and N. Opitz, „The pCO_2/pO_2 optrode: A new probe for measuring pCO_2 and pO_2 of gases and liquids", Zeitschrift für Naturforschung, 30C, pp. 532-533, 1975.
[23] B.D. MacCraith, G. O'Keefe, C. McDonagh and A.K. McEvoy, „LED-based fiber optic oxygen sensor using sol-gel coating", Electronic Letters, 30, pp. 888-892, 1994.
[24] A. Mills and Q. Chang, „Fluorescence Plastic Thin-Film Sensor for Carbon Dioxide", Analyst, 118, pp. 839-843, 1993.
[25] C. Munkholm, D. Walt, F. Milanovich and S. Klainer, „Polymer Modification of Fiber Optic Chemical Sensors as a Method for Enhancement of Signals for pH Measurement", Analytical Chemistry, 58, pp. 1427-1430, 1986.
[26] C. Munkholm, D.M. Walt and F.P. Milanovich, „A Fiber Optic Sensor for Carbon Dioxide", Talanta, 35, pp. 109-114, 1988.
[27] G.L. Robert-Baldo, M.J. Morris and R.H. Byrne, „Fiber-Optic pH sensor for seawater monitoring", SPIE, 798, pp. 294-301, 1987.
[28] D.B. Papkovsky, „New oxygen sensors and their application to biosensing", Sensors and Actuators B, 29, pp. 213-218, 1995.
[29] N.P. Revsbech and B.B. Jørgensen, in K.C. Marshall (ed.), Advances in Microbial Ecology, 9, Plenum Press, New York, „Microelectrodes: Their use in Microbial Ecology", pp. 293-352, 1986.
[30] Z. Rosenwzeig and R. Kopelman, „Development of a Submicrometer Optical Fiber Oxygen Sensor", Analytical Chemistry, 67, pp. 2650-2654, 1995.
[31] O. Stern and M. Volmer, „Über die Abklingzeit der Fluoreszenz", Physikalische Zeitschrift, 20, pp. 183-188, 1919.
[32] W. Tan, Z.-Y. Shi, S. Smith, D. Birnbaum and R. Kopelman, „Submicrometer Intracellular Chemical Optical Fiber Sensors", Science, 258, pp. 778-781, 1992.
[33] J.K. Tusa, T. Hacker, D.R. Hansmann, T.M. Kaput and T.P. Maxwell, „Fiber Optic Microsensor for Continous In-Vivo Measurement of Blood Gases", Proceedings SPIE, 713, pp. 137-143, 1986.
[34] J.M. Vanderkooi and D.F. Wilson, „A new method of measuring oxygen concentration in biological systems, Advancements of Experimental Medicine, 200, pp. 189-193, 1986.
[35] F. Wenzhöfer, O. Kohls and O. Holby, „Deep Penetration of Oxygen Measured in situ by Oxygen Optodes", Deep-Sea Research, (subm. f. publ.), 1998.
[36] O.S. Wolfbeis, „Fiber Optic Chemical Sensors and Biosensors", Vol. I+II, CRC Press, Boca Raton and London, 1991.
[37] Z. Zhang, K.T.V. Grattan and A.W. Palmer, „Phase-Locked Detection of Fluorescence Lifetime", Review of Scientific Instrumentation, 64 (9), pp. 2531-2540, 1993.